

APPENDIX I:

CLAIM AMENDMENTS:

Cancel Claims 1 to 10, 12 to 14 and 16 to 24, and enter new Claims 27 to 49 as indicated in the following listing of the claims:

1. - 26. (*canceled*)
27. (*new*) A process for the microbiological oxidation of a compound having an N- or S-heterocyclic mono- or polynuclear aromatic moiety, which process comprises oxidizing at least one aromatic C-H group of the heterocyclic aromatic moiety by
 - a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
 - a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium, andwherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having the amino acid sequence according to SEQ ID NO:2 by mutation, and wherein the mutation consists of at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88 of SEQ ID NO:2.
28. (*new*) A process as claimed in claim 27, wherein the exogenous or intermediately formed substrate of claim 27, alternative a1), or the substrate contained in the reaction medium of claim 27, alternative a2) is selected from optionally substituted N- or S-heterocyclic mono- or polynuclear aromatic compounds.
29. (*new*) A process as claimed in claim 27, wherein the mutation consists of at least one functional mutation in at least one of the sequence regions 73-82, 86-88 and 172-224.
30. (*new*) A process as claimed in claim 27, where the mutant has one of the following mono- or polyamino acid substitutions:
 - a) Phe87Val;
 - b) Phe87Val and Leu188Gln;

- c) Phe87Val, and Leu188Gln, and Ala74Gly.
31. (new) A process as claimed in claim 27, wherein the exogenous substrate is at least one compound selected from unsubstituted or substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds, the exogenous substrate is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.
32. (new) A process as claimed in claim 31, wherein the exogenous substrate is a compound selected from indole, 1-methylindole, acridine, 6-methyl- or 8-methylquinoline, quinoline and quinaldine.
33. (new) A process for the microbiological production of indigo and/or indirubin, which comprises
- a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or
 - a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium.
34. (new) A process as claimed in claim 33, wherein the indigo and/or indirubin obtained, which was produced by oxidation of intermediately formed indole, is isolated from the medium.
35. (new) A process as claimed in claim 34, wherein the indole oxidation is carried out by culturing the microorganisms in the presence of oxygen at a culturing temperature of approximately 20 to 40°C and a pH of approximately 6 to 9.
36. (new) A process as claimed in claim 34, where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2 by mutation, and wherein the mutation consists of at least one functional mutation in at least one of the amino acid sequence

regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88 of SEQ ID NO:2, including the substitution Phe87Val.

37. (new) A process as claimed in claim 36, where the monooxygenase has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val;
- b) Phe87Val, Leu188Gln; or
- c) Phe87Val, Leu188Gln, Ala74Gly.

38. (new) A cytochrome P450 monooxygenase which is capable of at least one of the following reactions:

- a) oxidation of optionally substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds;
- b) oxidation of optionally substituted mono- or polynuclear aromatics;
- c) oxidation of straight-chain or branched alkanes and alkenes;
- d) oxidation of optionally substituted cycloalkanes and cycloalkenes;

where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2 by mutation, and wherein the mutation consists of at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88 of SEQ ID NO:2; except the single mutant Phe87Val.

39. (new) A monooxygenase as claimed in claim 38, which has at least one functional mutation in at least one of the sequence regions 73-82, 86-88 and 172-224.

40. (new) A monooxygenase as claimed in claim 38, which has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val, Leu188Gln; or
- b) Phe87Val, Leu188Gln, Ala74Gly;

and functional equivalents thereof which are capable of at least one of the above oxidation reactions.

41. (new) A nucleic acid sequence coding for a monooxygenase according to claim 38.

42. (new) An expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a coding sequence which comprises a nucleic acid sequence according to claim 41.
43. (new) A vector comprising at least one expression construct according to claim 42.
44. (new) A recombinant microorganism transformed by at least one vector as claimed in claim 43.
45. (new) A microorganism as claimed in claim 44, selected from bacteria of the genus *Escherichia*.
46. (new) A process for microbiological oxidation of optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises
- a1) culturing a recombinant cytochrome P450-producing microorganism as claimed in claim 44 in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
 - a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2 by mutation, and wherein the mutation consists of at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88 of SEQ ID NO:2; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium;
- where the monooxygenase mutant Phe87Val is not excluded.
47. (new) A process as claimed in claim 46, wherein the exogenous or intermediately formed substrate is selected from:
- a) optionally substituted mono- or polynuclear aromatics;
 - b) straight-chain or branched alkanes and alkenes;
 - c) optionally substituted cycloalkanes and cycloalkenes.
48. (new) A process as claimed in claim 46, where the cytochrome P450 monooxygenase has at least one of the following mono- or poly-amino acid substitutions:
- a) Phe87Val;

- b) Phe87Val, Leu188Gln; or
 - c) Phe87Val, Leu188Gln, Ala74Gly.
49. (new) A bioreactor comprising the cytochrome P450 monooxygenase as claimed in claim 37 or a recombinant microorganism transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrome P450 monooxygenase of claim 37 in immobilized form.